

## WE CLAIM

1. Peptide nucleic acid probe for detecting a target sequence of one or more mycobacteria optionally present in a sample, said probe being capable of hybridising to a target sequence of mycobacterial rDNA, precursor rRNA or rRNA forming detectable hybrids,  
 5 and a mixture of such probes.
2. Peptide nucleic acid probe according to claim 1, said probe being capable of hybridising to a target sequence of mycobacterial rDNA, precursor rRNA, or 23S, 16S or 5S rRNA forming  
 10 detectable hybrids,  
 and a mixture of such probes.
3. Peptide nucleic acid probe according to claim 1, said probe being capable of hybridising to a target sequence of mycobacterial rDNA, precursor rRNA, or 23S, 16S or 5S rRNA forming  
 15 detectable hybrids, said target sequence being obtainable by
  - (a) comparing the nucleobase sequences of said mycobacterial rRNA or rDNA of one or more mycobacteria to be detected with the corresponding nucleobase sequence of organism(s), in particular other mycobacteria, from which said one or more  
 20 mycobacteria are to be distinguished,
  - (b) selecting a target sequence of said rRNA or rDNA which includes at least one nucleobase differing from the corresponding nucleobase of the organism(s), in particular other mycobacteria, from which said one or more mycobacteria are to be distinguished, and  
 25
  - (c) determining the capability of said probe to hybridise to the selected target sequence to form detectable hybrids,  
 and a mixture of such probes.
- 30 4. Peptide nucleic acid probe according to claim 1, said probe being capable of hybridising to a target sequence of mycobacterial rDNA, precursor rRNA or 23S, 16S or 5S rRNA forming detectable hybrids, said probe being obtainable by
  - (a) comparing the nucleobase sequences of said mycobacterial rRNA or rDNA of one or more  
 35 mycobacteria to be detected with the corresponding nucleobase sequence of organism(s), in particular other mycobacteria, from which said one or more mycobacteria are to be distinguished,

(b) selecting a target sequence of said rRNA or rDNA which includes at least one nucleobase differing from the corresponding nucleobase of the organism(s), in particular other mycobacteria, from which said one or more mycobacteria are to be distinguished,

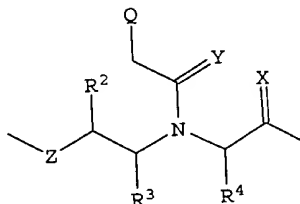
5 (c) synthesising said probe, and

(d) determining the capability of said probe to hybridise to the selected target sequence to form detectable hybrids,  
and a mixture of such probes.

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5. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC) or for detecting a target sequence of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex (MOTT) optionally present in a sample, which probe comprises from 6 to 30 polymerised peptide nucleic acid moieties, said probe being capable of hybridising to a target sequence of mycobacterial rDNA, precursor rRNA or 23S, 16S or 5S rRNA forming detectable hybrids,  
and a mixture of such probes.

20 6. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of rDNA, precursor rRNA or 23S, 16S or 5S rRNA of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC) or for detecting a target sequence of rDNA, precursor rRNA or 23S, 16S or 5S rRNA of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex (MOTT) optionally present in a sample, which probe  
25 comprises from 10 to 30 polymerised moieties of formula (I)



(I)

30

wherein each X and Y independently designate O or S,

each Z independently designates O, S, NR¹, or C(R¹)₂, wherein each R¹ independently designate H, C₁-₆ alkyl, C₁-₆ alkenyl, C₁-₆ alkynyl,

35 each R², R³ and R⁴ designate independently H, the side chain of a naturally occurring amino acid, the side chain of a non-naturally occurring amino acid, C₁-₄ alkyl, C₁-₄ alkenyl or C₁-₄ alkynyl, or a functional group, each Q independently designates a naturally occurring nucleobase, a non-naturally occurring nucleobase, an intercalator, a nucleobase-binding

group, a label or H,

with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with the target sequence of said mycobacterial rDNA, precursor rRNA or 23S, 16S or  
 5 5S rRNA,  
 and a mixture of such probes.

7. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 23S  
 rRNA of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC)  
 10 optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of  
 formula (I) as defined in claim 6,

with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of  
 which a subsequence includes at least one nucleobase that is complementary to a  
 15 nucleobase of *M. tuberculosis* 23S rRNA differing from the corresponding nucleobase of at  
 least *M. avium* located within the following domains

Positions 149-158 in Figure 1A,  
 Positions 220-221 in Figure 1A,  
 20 Positions 328-361 in Figure 1A and Figure 1B,  
 Positions 453-455 in Figure 1B,  
 Positions 490-501 in Figure 1B,  
 Positions 637-660 in Figure 1C,  
 Positions 706-712 in Figure 1D,  
 25 Positions 762-789 in Figure 1D,  
 Position 989 in Figure 1D,  
 Positions 1068-1072 in Figure 1D,  
 Position 1148 in Figure 1E,  
 Positions 1311-1329 in Figure 1E,  
 30 Positions 1361-1364 in Figure 1F,  
 Position 1418 in Figure 1F,  
 Positions 1563-1570 in Figure 1F,  
 Positions 1627-1638 in Figure 1G,  
 Positions 1675-1677 in Figure 1G,  
 35 Position 1718 in Figure 1G,  
 Positions 1734-1740 in Figure 1H,  
 Positions 1967-1976 in Figure 1H,  
 Positions 2403-2420 in Figure 1H,

Positions 2457-2488 in Figure 1I,  
 Positions 2952-2956 in Figure 1I,  
 Positions 2966-2969 in Figure 1J,  
 Positions 3000-3003 in Figure 1J or  
 5 Positions 3097-3106 in Figure 1J,

and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with a target sequence of said mycobacterial 23S rRNA, and a mixture of such probes.

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8. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 16S rRNA of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,

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with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of which a subsequence includes at least one nucleobase that is complementary to a nucleobase of *M. tuberculosis* 16S rRNA differing from the corresponding nucleobase of at least *M. avium* located within the following domains

20

Positions 76-79 in Figure 2A,  
 Positions 98-101 in Figure 2A,  
 Positions 135-136 in Figure 2A,  
 Positions 194-201 in Figure 2B,  
 25 Positions 222-229 in Figure 2B,  
 Position 242 in Figure 2B,  
 Position 474 in Figure 2C,  
 Positions 1136-1145 in Figure 2C,  
 Positions 1271-1272 in Figure 2C,  
 30 Positions 1287-1292 in Figure 2D,  
 Position 1313 in Figure 2D, or  
 Position 1334 in Figure 2D,

35

and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with a target sequence of said mycobacterial 16S rRNA, and a mixture of such probes.

9. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 5S rRNA

of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,

- 5 with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of which a subsequence includes at least one nucleobase that is complementary to a nucleobase of *M. tuberculosis* 5S rRNA differing from the corresponding nucleobase of at least *M. avium* located within the following domain

10 Positions 86-90 in Figure 3

and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with a target sequence of said mycobacterial 5S rRNA, and a mixture of such probes.

15

10. Peptide nucleic acid probe according to claim 7 or 8 for detecting a target sequence of 23S or 16S rRNA of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,

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with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of which a subsequence includes at least one nucleobase that is complementary to a nucleobase of *M. tuberculosis* 23S or 16 S rRNA differing from the corresponding nucleobase of at least *M. avium* located within the following domains

25

Positions 149-158 in Figure 1A,  
Positions 328-361 in Figure 1A and Figure 1B,  
Positions 490-501 in Figure 1B,  
Positions 637-660 in Figure 1C,

30

Positions 762-789 in Figure 1D,  
Positions 1068-1072 in Figure 1D,  
Positions 1311-1329 in Figure 1E,  
Positions 1361-1364 in Figure 1F,  
Positions 1563-1570 in Figure 1F,

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Positions 1627-1638 in Figure 1G,  
Positions 1734-1740 in Figure 1H,  
Positions 2457-2488 in Figure 1I,  
Positions 2952-2956 in Figure 1I,

Positions 3097-3106 in Figure 1J,  
 Positions 135-136 in Figure 2 A, or  
 Positions 1287-1292 in Figure 2D,

- 5 and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with a target sequence of said mycobacterial 23S or 16S rRNA, and a mixture of such probes.

11. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 23S  
 10 rRNA of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex (MOTT) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,

- with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of  
 15 which a subsequence includes at least one nucleobase that is complementary to a nucleobase of *M. avium* 23S rRNA differing from the corresponding nucleobase of at least *M. tuberculosis* located within the following domains

- Positions 99-101 in Figure 4A,  
 20 Position 183 in Figure 4A,  
 Positions 261-271 in Figure 4A,  
 Positions 281-284 in Figure 4B,  
 Positions 290-293 in Figure 4B,  
 Positions 327-335 in Figure 4B,  
 25 Positions 343-357 in Figure 4B,  
 Positions 400-405 in Figure 4B and Figure 4C,  
 Positions 453-462 in Figure 4C,  
 Positions 587-599 in Figure 4C,  
 Positions 637-660 in Figure 4D,  
 30 Positions 704-712 in Figure 4D,  
 Positions 763-789 in Figure 4E,  
 Positions 1060-1074 in Figure 4E,  
 Positions 1177-1185 in Figure 4E,  
 Positions 1259-1265 in Figure 4F,  
 35 Positions 1311-1327 in Figure 4F,  
 Positions 1345-1348 in Figure 4F,  
 Positions 1361-1364 in Figure 4G,  
 Positions 1556-1570 in Figure 4G,

- Positions 1608-1613 in Figure 4H,  
 Positions 1626-1638 in Figure 4H,  
 Positions 1651-1659 in Figure 4H,  
 Positions 1675-1677 in Figure 4H,  
 5 Positions 1734-1741 in Figure 4H,  
 Positions 1847-1853 in Figure 4I,  
 Positions 1967-1976 in Figure 4I,  
 Positions 2006-2010 in Figure 4I,  
 Positions 2025-2027 in Figure 4I,  
 10 Positions 2131-2132 in Figure 4J,  
 Positions 2252-2255 in Figure 4J,  
 Positions 2396-2405 in Figure 4J and Figure 4K,  
 Positions 2416-2420 in Figure 4K,  
 Positions 2474-2478 in Figure 4K,  
 15 Position 2687 in Figure 4K,  
 Position 2719 in Figure 4K,  
 Position 2809 in Figure 4L,  
 Positions 3062-2068 in Figure 4L, or  
 Positions 3097-3106 in Figure 4L,  
 20
- and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with a target sequence of said mycobacterial 23S rRNA, and a mixture of such probes.
- 25 12. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 16S rRNA of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex (MOTT) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,
- 30 with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of which a subsequence includes at least one nucleobase that is complementary to a nucleobase of *M. avium* 16S rRNA differing from the corresponding nucleobase of at least *M. tuberculosis* located within the following domains
- 35 Positions 135-136 in Figure 5A,  
 Positions 472-475 in Figure 5A,  
 Positions 1136-1144 in Figure 5A,  
 Positions 1287-1292 in Figure 5B,

Position 1313 in Figure 5B, or  
Position 1334 in Figure 5B,

and further with the proviso that the probe comprising such subsequence is capable of forming  
5 detectable hybrids with a target sequence of said mycobacterial 16S rRNA,  
and a mixture of such probes.

13. Peptide nucleic acid probe according to claim 11 or 12 for detecting a target sequence of  
23S or 16S rRNA of one or more mycobacteria other than mycobacteria of the Mycobacterium  
10 tuberculosis Complex (MOTT) optionally present in a sample, which probe comprises from 10  
to 30 polymerised moieties of formula (I) as defined in claim 6,

with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of  
which a subsequence includes at least one nucleobase that is complementary to a  
15 nucleobase of *M. avium* 23S or 16S rRNA differing from the corresponding nucleobase of at  
least *M. tuberculosis* located within the following domains

Positions 99-101 in Figure 4A,  
Positions 290-293 in Figure 4B,  
20 Positions 400-405 in Figure 4B and Figure 4C,  
Positions 453-462 in Figure 4C,  
Positions 637-660 in Figure 4D,  
Positions 763-789 in Figure 4E,  
Positions 1311-1327 in Figure 4F,  
25 Positions 1361-1364 in Figure 4G,  
Positions 1734-1741 in Figure 4H,  
Positions 2025-2027 in Figure 4I,  
Positions 2474-2478 in Figure 4K,  
Positions 3062-2068 in Figure 4L, or  
30 Positions 1287-1292 in Figure 5B,

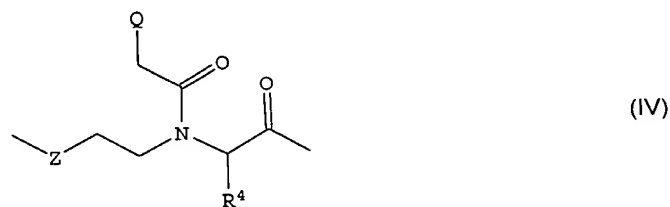
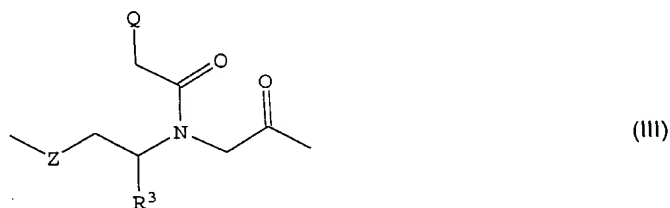
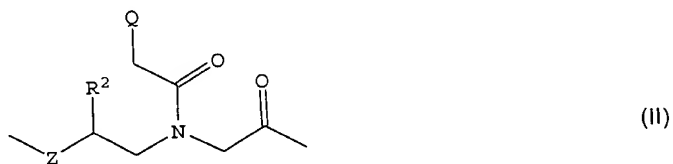
and further with the proviso that the probe comprising such subsequence is capable of forming  
detectable hybrids with a target sequence of said mycobacterial 23S or 16S rRNA,  
and a mixture of such probes.

35 14. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of 23S,  
16S or 5S rRNA of one or more mycobacteria of the Mycobacterium tuberculosis Complex  
(MTC) or for detecting a target sequence of 23S, 16S or 5S rRNA of one or more



mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex (MOTT) optionally present in a sample, which probe comprises from 10 to 30 polymerised moieties of formula (I) as defined in claim 6,

- 5 with the proviso that the Qs of adjacent moieties are selected so as to form a sequence of which a subsequence includes at least one nucleobase that is complementary to a nucleobase that differs from the corresponding nucleobase of 23S, 16S or 5S rRNA of said one or more mycobacteria located within the following domains
- 10 positions 2568-2569 in Figure 6,
- Position 452 in Figure 7,
- Positions 473-477 in Figure 7, or
- Positions 865-866 in Figure 7,
- 15 and further with the proviso that the probe comprising such subsequence is capable of forming detectable hybrids with the target sequence of said mycobacterial 23S, 16S or 5S rRNA, and a mixture of such probes.
- 20 15. Peptide nucleic acid probe according to claim 6 of formula (II), (III), or (IV)



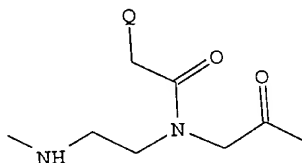
wherein Z, R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup>, and Q is as defined in claim 6 with the provisos defined in claims 6 to 14,

and a mixture of such probes.

16. Peptide nucleic acid probe according to claim 6, wherein Z is NH, NCH<sub>3</sub> or O, each R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> independently designate H or the side chain of a naturally occurring amino acid, the side chain of a non-naturally occurring amino acid, or C<sub>1-4</sub> alkyl, and each Q is a naturally occurring nucleobase or a non-naturally occurring nucleobase with the provisos defined in claims 6 to 14, and a mixture of such probes.

17. Peptide nucleic acid probe according to claim 6, wherein Z is NH or O, and R<sup>2</sup> is H or the side chain of Ala, Asp, Cys, Glu, His, HomoCys, Lys, Orn, Ser or Thr, and Q is a nucleobase selected from thymine, adenine, cytosine, guanine, uracil, iso-C and 2,6-diaminopurine with the provisos defined in claims 6 to 14, and a mixture of such probes.

18. Peptide nucleic acid probe according to claim 6 of formula (V)



(V)

wherein R<sup>4</sup> is H or the side chain of Ala, Asp, Cys, Glu, His, HomoCys, Lys, Orn, Ser or Thr, and Q is as defined in claim 17 with the provisos defined in claims 6 to 14, and a mixture of such probes.

19. Peptide nucleic acid probe according to claim 1 further comprising one or more labels and a mixture of such probes, which labels may be mutually identical or different, which probes optionally may comprise one or more linkers, and which probes may be mutually identical or different with the provisos defined in claims 6 to 14.

20. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of one or more mycobacteria, the nucleobase sequence of said probe being substantially complementary to the nucleobase sequence of said target sequence.

21. Peptide nucleic acid probe according to claim 1 for detecting a target sequence of one or more mycobacteria, the nucleobase sequence of said probe being complementary to the nucleobase sequence of said target sequence.

22. Peptide nucleic acid probes according to claim 6, wherein the Qs of adjacent moieties are selected so as to form the following subsequences

- |    |   |                        |
|----|---|------------------------|
|    | AGA TGC GGG TAG CAC (selected from positions 149-158 in Figure 1A),               | (Seq ID no 1)          |
| 5  | TGT TTT CTC CTC CTA (selected from positions 220-221 in Figure 1A),               | (Seq ID no 2)          |
|    | ACT GCC TCT CAG CCG (selected from positions 328-361 in Figure 1A and Figure 1B), | (Seq ID no 3)          |
|    | TGA TAC TAG GCA GGT (selected from positions 453-455 in Figure 1B),               | (Seq ID no 4)          |
|    | CGG ATT CAC AGC GGA (selected from positions 490-501 in Figure 1B),               | (Seq ID no 5)          |
| 10 | TCA CCA CCC TCC TCC (selected from positions 637-660 in Figure 1C),               | (Seq ID no 6)          |
|    | CCA CCC TCC TCC (selected from positions 637-660 in Figure 1C),                   | (modified Seq ID no 6) |
|    | TTA ACC TTG CGA CAT (selected from positions 706-712 in Figure 1C),               | (Seq ID no 7)          |
|    | ACT ATT CAC ACG CGC (selected from positions 762-789 in Figure 1D),               | (Seq ID no 8)          |
|    | CTC CGC GGT GAA CCA (selected from position 989 in Figure 1D),                    | (Seq ID no 9)          |
| 15 | GCT TTA CAC CAC GGC (selected from positions 1068-1072 in Figure 1D),             | (Seq ID no 10)         |
|    | ACG CTT GGG GGC CTT (selected from position 1148 in Figure 1E),                   | (Seq ID no 11)         |
|    | CCA CAC CCA CCA CAA (selected from positions 1311-1329 in Figure 1E),             | (Seq ID no 12)         |
|    | CCG GTG GCT TCG CTG (selected from positions 1361-1364 in Figure 1F),             | (Seq ID no 13)         |
|    | ACT TGC CTT GTC GCT (selected from position 1418 in Figure 1F),                   | (Seq ID no 14)         |
| 20 | GAT TCG TCA CGG GCG (selected from positions 1563-1570 in Figure 1F),             | (Seq ID no 15)         |
|    | AAC TCC ACA CCC CCG (selected from positions 1627-1638 in Figure 1G),             | (Seq ID no 16)         |
|    | ACT CCA CAC CCC CGA (selected from positions 1627-1638 in Figure 1G),             | (Seq ID no 17)         |
|    | ACC CCT TCG CTT GAC (selected from positions 1675-1677 in Figure 1G),             | (Seq ID no 18)         |
|    | CTT GCC CCA GTG TTA (selected from position 1718 in Figure 1G),                   | (Seq ID no 19)         |
| 25 | CTC TCC CTA CCG GCT (selected from positions 1734-1740 in Figure 1H),             | (Seq ID no 20)         |
|    | GAT ATT CCG GTC CCC (selected from positions 1967-1976 in Figure 1H),             | (Seq ID no 21)         |
|    | ACT CCG CCC CAA CTG (selected from positions 2403-2420 in Figure 1H),             | (Seq ID no 22)         |
|    | CTG TCC CTA AAC CCG (selected from positions 2457-2488 in Figure 1I),             | (Seq ID no 23)         |
|    | TTC GAG GTT AGA TGC (selected from positions 2457-2488 in Figure 1I),             | (Seq ID no 24)         |
| 30 | GTC CCT AAA CCC GAT (selected from positions 2457-2488 in Figure 1I),             | (Seq ID no 25)         |
|    | GGT GCA CCA GAG GTT (selected from positions 2952-2956 in Figure 1I),             | (Seq ID no 26)         |
|    | CTG GCG GGA CAA CTG (selected from positions 2966-2969 in Figure 1J),             | (Seq ID no 27)         |
|    | TTA TCC TGA CCG AAC (selected from positions 3000-3003 in Figure 1J),             | (Seq ID no 28)         |
|    | GAC CTA TTG AAC CCG (selected from positions 3097-3106 in Figure 1J),             | (Seq ID no 29)         |
| 35 | GAA GAG ACC TTT CCG (selected from positions 76-79 in Figure 2A),                 | (Seq ID no 30)         |
|    | CAC TCG AGT ATC TCC (selected from positions 98-101 in Figure 2A),                | (Seq ID no 31)         |
|    | ATC ACC CAC GTG TTA (selected from positions 136-136 in Figure 2A),               | (Seq ID no 32)         |
|    | GCA TCC CGT GGT CCT (selected from positions 194-201 in Figure 2B),               | (Seq ID no 33)         |
| 40 | CAC AAG ACA TGC ATC (selected from positions 194-201 in Figure 2B),               | (Seq ID no 34)         |
|    | TAA AGC GCT TTC CAC (selected from positions 222-229 in Figure 2B),               | (Seq ID no 35)         |
|    | GCT CAT CCC ACA CCG (selected from position 242 in Figure 2B),                    | (Seq ID no 36)         |

	CCG AGA GAA CCC GGA (selected from position 474 in Figure 2C),	(Seq ID no 37)
	AGT CCC CAC CAT TAC (selected from positions 1136-1145 in Figure 2C),	(Seq ID no 38)
	AAC CTC GCG GCA TCG (selected from positions 1271-1272 in Figure 2C),	(Seq ID no 39)
	GGC TTT TAA GGA TTC (selected from positions 1287-1292 in Figure 2D),	(Seq ID no 40)
5	GAC CCC GAT CCG AAC (selected from position 1313 in Figure 2D),	(Seq ID no 41)
	CCG ACT TCA CGG GGT (selected from position 1334 in Figure 2D),	(Seq ID no 42)
	CGG AGG GGC AGT ATC (selected from positions 86-90 in Figure 3),	(Seq ID no 43)
10	GAT CAA TGC TCG GTT (selected from positions 99-101 in Figure 4A),	(Seq ID no 44)
	TTC CCC GCG TTA CCT (selected from position 183 in Figure 4A),	(Seq ID no 45)
	TTA GCC TGT TCC GGT (selected from positions 261-271 in Figure 4A),	(Seq ID no 46)
	GCA TGC GGT TTA GCC (selected from positions 281-284 in Figure 4B),	(Seq ID no 47)
	TAC CCG GTT GTC CAT (selected from positions 290-293 in Figure 4B),	(Seq ID no 48)
15	GTA GAG CTG AGA CAT (selected from positions 327-335 and 343-357 in Figure 4B),	(Seq ID no 49)
	GCC GTC CCA GGC CAC (selected from positions 400-405 in Figure 4B and Figure 4C),	(Seq ID no 50)
	CTC GGG TGT TGA TAT (selected from positions 453-462 in Figure 4C),	(Seq ID no 51)
20	ACT ATT TCA CTC CCT (selected from positions 587-599 in Figure 4C),	(Seq ID no 52)
	ACG CCA TCA CCC CAC (selected from positions 637-660 in Figure 4D),	(Seq ID no 53)
	CGA CGT GTC CCT GAC (selected from positions 704-712 in Figure 4D),	(Seq ID no 54)
	ACT ACA CCC CAA AGG (selected from positions 763-789 in Figure 4E),	(Seq ID no 55)
	CAC GCT TTT ACA CCA (selected from positions 1060-1074 in Figure 4E),	(Seq ID no 56)
25	GCG ACT ACA CAT CCT (selected from positions 1177-1185 in Figure 4E),	(Seq ID no 57)
	CGG CGC ATA ATC ACT (selected from positions 1259-1265 in Figure 4F),	(Seq ID no 58)
	CCA CAT CCA CCG TAA (selected from positions 1311-1327 in Figure 4F),	(Seq ID no 59)
	CGC TGA ATG GGG GAC (selected from positions 1345-1348 in Figure 4F),	(Seq ID no 60)
	GGA GCT TCG CTG AAT (selected from positions 1361-1364 in Figure 4G),	(Seq ID no 61)
30	CGG TCA CCC GGA GCT (selected from positions 1361-1364 in Figure 4G),	(Seq ID no 62)
	GGA CGC CCA TAC ACG (selected from positions 1556-1570 in Figure 4G),	(Seq ID no 63)
	GAA GGG GAA TGG TCG (selected from positions 1608-1613 in Figure 4H),	(Seq ID no 64)
	AAT CGC CAC GCC CCC (selected from positions 1626-1638 in Figure 4H),	(Seq ID no 65)
	CAG CGA AGG TCC CAC (selected from positions 1651-1659 in Figure 4H),	(Seq ID no 66)
35	GTC ACC CCA TTG CTT (selected from positions 1675-1677 in Figure 4H),	(Seq ID no 67)
	ATC GCT CTC TAC GGG (selected from positions 1734-1741 in Figure 4H),	(Seq ID no 68)
	GTG TAT GTG CTC GCT (selected from positions 1847-1853 in Figure 4I),	(Seq ID no 69)
	ACG GTA TTC CGG GCC (selected from positions 1967-1976 in Figure 4I),	(Seq ID no 70)
	GGC CGA ATC CCG CTC (selected from positions 2006-2010 in Figure 4I),	(Seq ID no 71)
40	AAA CAG TCG CTA CCC (selected from positions 2025-2027 in Figure 4I),	(Seq ID no 72)
	CCT TAC GGG TTA ACG (selected from positions 2131-2132 in Figure 4J),	(Seq ID no 73)
	GAG ACA GTT GGG AAG (selected from positions 2252-2255 in Figure 4J),	(Seq ID no 74)
	TGG CGT CTG TGC TTC (selected from positions 2396-2405 in	

	Figure 4J and Figure 4K),	(Seq ID no 75)
	CGA CTC CAC ACA AAC (selected from positions 2416-2420 in Figure 4K),	(Seq ID no 76)
	GAT AAG GGT TCG ACG (selected from positions 2474-2478 in Figure 4K),	(Seq ID no 77)
	ATC CGT TGA GTG ACA (selected from position 2687 in Figure 4K),	(Seq ID no 78)
5	CAG CCC GTT ATC CCC (selected from position 2719 in Figure 4K),	(Seq ID no 79)
	AAC CTT TGG GAC CTG (selected from position 2809 in Figure 4L),	(Seq ID no 80)
	TAA AAG GGT GAG AAA (selected from positions 3062-3068 in Figure 4L),	(Seq ID no 81)
	GTC TGG CCT ATC AAT (selected from positions 3097-3106 in Figure 4L),	(Seq ID no 82)
10	AGA TTG CCC ACG TGT (selected from positions 135-136 in Figure 5A),	(Seq ID no 83)
	AAT CCG AGA AAA CCC (selected from positions 472-475 in Figure 5A),	(Seq ID no 84)
	GCA TTA CCC GCT GGC (selected from positions 1136-1144 in Figure 5B),	(Seq ID no 85)
	TTA AAA GGA TTC GCT (selected from positions 1287-1292 in Figure 5B),	(Seq ID no 86)
	AGA CCC CAA TCC GAA (selected from position 1313 in Figure 5B),	(Seq ID no 87)
15	GAC TCC GAC TTC ATG (selected from position 1334 in Figure 5B),	(Seq ID no 88)
	GTC TTT TCG TCC TGC (selected from positions 2568-2569 in Figure 6),	(Seq ID no 89)
	GTC TTA TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 90)
	GTC TTC TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 91)
20	GTC TTG TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 92)
	GTC TAT TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 93)
	GTC TCT TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 94)
	GTC TGT TCG TCC TGC (selected from positions 2568 in Figure 6),	(Seq ID no 95)
25	TTG GCC GGT GCT TCT (selected from positions 452 in Figure 7),	(Seq ID no 96)
	TTG GCC GGT ACT TCT (selected from positions 452 in Figure 7),	(Seq ID no 97)
	TTG GCC GGT CCT TCT (selected from positions 452 in Figure 7),	(Seq ID no 98)
	TTG GCC GGT TCT TCT (selected from positions 452 in Figure 7),	(Seq ID no 99)
	ACC GCG GCT GCT GGC (selected from positions 473-477 in Figure 7),	(Seq ID no 100)
30	ACC GCG GCT ACT GGC (selected from positions 473 in Figure 7),	(Seq ID no 101)
	ACC GCG GCT CCT GGC (selected from positions 473 in Figure 7), or	(Seq ID no 102)
	ACC GCG GCT TCT GGC (selected from positions 473 in Figure 7),	(Seq ID no 103)
	CGG CAG CTG GCA CGT (selected from positions 474 in Figure 7),	(Seq ID no 104)
	CGG CCG CTG GCA CGT (selected from positions 474 in Figure 7),	(Seq ID no 105)
35	CGG CTG CTG GCA CGT (selected from positions 474 in Figure 7),	(Seq ID no 106)
	CGT ATT ACC GCA GCT (selected from positions 477 in Figure 7),	(Seq ID no 107)
	CGT ATT ACC GCC GCT (selected from positions 477 in Figure 7),	(Seq ID no 107)
	CGT ATT ACC GCT GCT (selected from positions 477 in Figure 7),	(Seq ID no 109)
	TTC CTT TGA GTT TTA (selected from positions 865-866 in Figure 7),	(Seq ID no 110)
40	TTC CTT TAA GTT TTA (selected from positions 865 in Figure 7),	(Seq ID no 111)
	TTC CTT TCA GTT TTA (selected from positions 865 in Figure 7),	(Seq ID no 112)
	TTC CTT TTA GTT TTA (selected from positions 865 in Figure 7),	(Seq ID no 113)
	TTC CTT AGA GTT TTA (selected from positions 866 in Figure 7),	(Seq ID no 114)

	TTC CTT CGA GTT TTA (selected from positions 866 in Figure 7),	(Seq ID no 115)
	TTC CTT GGA GTT TTA (selected from positions 866 in Figure 7),	(Seq ID no 116)
	CAT GTG TCC TGT GGT	(Seq ID no 117)
	CGT CAG CCC GAG AAA	(Seq ID no 118)
5	CAC TAC ACA CGC TCG	(Seq ID no 119)
	TGG CGT TGA GGT TTC and	(Seq ID no 120)
	AAC ACT CCC TTT GGA	(Seq ID no 123)
	and a mixture of such probes.	
10	23. Peptide nucleic acid probes according to claim 22, wherein the Qs of adjacent moieties are selected so as to form the following subsequences	
	TCA CCA CCC TCC TCC	(Seq ID no 6)
15	CCA CCC TCC TCC	(modified Seq ID no 6)
	ACT ATT CAC ACG CGC	(Seq ID no 8)
	CCA CAC CCA CCA CAA	(Seq ID no 12)
	AAC TCC ACA CCC CCG	(Seq ID no 16)
	ACT CCA CAC CCC CGA	(Seq ID no 17)
20	ACT CCG CCC CAA CTG	(Seq ID no 22)
	CTG TCC CTA AAC CCG	(Seq ID no 23)
	TTC GAG GTT AGA TGC	(Seq ID no 24)
	GTC CCT AAA CCC GAT	(Seq ID no 25)
	GAC CTA TTG AAC CCG	(Seq ID no 29)
25	GCA TCC CGT GGT CCT	(Seq ID no 33)
	CAC AAG ACA TGC ATC	(Seq ID no 34)
	GGC TTT TAA GGA TTC	(Seq ID no 40)
30	GAT CAA TGC TCG GTT	(Seq ID no 44)
	CGA CTC CAC ACA AAC	(Seq ID no 76)
	GCA TTA CCC GCT GGC	(Seq ID no 85)
35	GTC TTA TCG TCC TGC	(Seq ID no 90)
	GTC TTC TCG TCC TGC	(Seq ID no 91)
	GTC TTG TCG TCC TGC	(Seq ID no 92)
	GTC TAT TCG TCC TGC	(Seq ID no 93)
	GTC TCT TCG TCC TGC	(Seq ID no 94)
40	GTC TGT TCG TCC TGC	(Seq ID no 95)
	AAC ACT CCC TTT GGA	(Seq ID no 123)

CAT GTG TCC TGT GGT  
CGT CAG CCC GAG AAA

(Seq ID no 117)

(Seq ID no 118)

- 5 CAC TAC ACA CGC TCG,  
TGG CGT TGA GGT TTC

(Seq ID no 119)

(Seq ID no 120)

and a mixture of such probes.

- 10 24. Peptide nucleic acid probes according to claim 22 selected from

- |    |   |                                |
|----|---|--------------------------------|
|    | Lys(Flu)-Lys(Flu)-TCA CCA CCC TCC TCC-NH <sub>2</sub>     | (OK 446/modified Seq ID no 6)  |
|    | Lys(Flu)-Lys(Flu)-CCA CCC TCC TCC-NH <sub>2</sub>         | (OK 575/modified Seq ID no 6)  |
|    | Lys(Flu)-Lys(Flu)-ACT ATT CAC ACG CGC-NH <sub>2</sub>     | (OK 447/modified Seq ID no 8)  |
| 15 | Lys(Flu)-ACT ATT CAC ACG CGC-NH <sub>2</sub>              | (OK 688/modified Seq ID no 8)  |
|    | Lys(Flu)-Lys(Flu)-CCA CAC CCA CCA CAA-NH <sub>2</sub>     | (OK 448/modified Seq ID no 12) |
|    | Lys(Flu)-Lys(Flu)-AAC TCC ACA CCC CCG-NH <sub>2</sub>     | (OK 449/modified Seq ID no 16) |
|    | Lys(Flu)-Lys(Flu)-ACT CCA CAC CCC CGA-NH <sub>2</sub>     | (OK 309/modified Seq ID no 17) |
|    | Lys(Flu)-Lys(Flu)-ACT CCG CCC CAA CTG-NH <sub>2</sub>     | (OK 450/modified Seq ID no 22) |
| 20 | Lys(Flu)-Lys(Flu)-CTG TCC CTA AAC CCG-NH <sub>2</sub>     | (OK 305/modified Seq ID no 23) |
|    | Lys(Flu)-Lys(Flu)-TTC GAG GTT AGA TGC-NH <sub>2</sub>     | (OK 306/modified Seq ID no 24) |
|    | H-Lys(Flu)-TTC GAG GTT AGA TGC-NH <sub>2</sub>            | (OK 682/modified Seq ID no 24) |
|    | Lys(Flu)-Lys(Flu)-GTC CCT AAA CCC GAT-NH <sub>2</sub>     | (OK 307/modified Seq ID no 25) |
|    | Lys(Flu)-GTC CCT AAA CCC GAT-NH <sub>2</sub>              | (OK 654/modified Seq ID no 25) |
| 25 | H-Lys(Flu)-GAC CTA TTG AAC CCG-NH <sub>2</sub>            | (OK 660/modified Seq ID no 29) |
|    | Lys(Flu)-Lys(Flu)-Gly-GCA TCC CGT GGT CCT-NH <sub>2</sub> | (OK 223/modified Seq ID no 33) |
|    | Lys(Flu)-Lys(Flu)-CAC AAG ACA TGC ATC-NH <sub>2</sub>     | (OK 310/modified Seq ID no 34) |
|    | Lys(Flu)-CAC AAG ACA TGC ATC-NH <sub>2</sub>              | (OK 655/modified Seq ID no 34) |
| 30 | H-Lys(Flu)-GGC TTT TAA GGA TTC-NH <sub>2</sub>            | (OK 689/modified Seq ID no 40) |
|    | H-Lys(Rho)-GGC TTT TAA GGA TTC-NH <sub>2</sub>            | (OK 689/modified Seq ID no 40) |
|    | Flu-β-Ala-β-Ala-GAT CAA TGC TCG GTT-NH <sub>2</sub>       | (OK 624/modified Seq ID no 44) |
|    | Flu-β-Ala-β-Ala-CGA CTC CAC ACA AAC-NH <sub>2</sub>       | (OK 612/modified Seq ID no 76) |
| 35 | Flu-β-Ala-β-Ala-GCA TTA CCC GCT GGC-NH <sub>2</sub>       | (OK 623/modified Seq ID no 85) |
|    | Lys(Flu)-GTC TTT TCG TCC TGC-NH <sub>2</sub>              | (OK 745/modified Seq ID no 89) |
|    | Lys(Rho)-GTC TTA TCG TCC TGC-NH <sub>2</sub>              | (OK 746/modified Seq ID no 90) |
| 40 | Lys(Rho)-GTC TTC TCG TCC TGC-NH <sub>2</sub>              | (OK 746/modified Seq ID no 91) |
|    | Lys(Rho)-GTC TTG TCG TCC TGC-NH <sub>2</sub>              | (OK 746/modified Seq ID no 92) |
|    | Lys(Rho)-GTC TAT TCG TCC TGC-NH <sub>2</sub>              | (OK 747/modified Seq ID no 93) |

Lys(Rho)-GTC TCT TCG TCC TGC-NH<sub>2</sub> (OK 747/modified Seq ID no 94)  
 Lys(Rho)-GTC TGT TCG TCC TGC-NH<sub>2</sub> (OK 747/modified Seq ID no 95)

Lys(Flu)-AAC ACT CCC TTT GGA-NH<sub>2</sub> (OK 749/modified Seq ID no 123)

5

wherein Flu denotes a 5-(and 6)-carboxyfluorescein label and Rho denotes a rhodamine label,  
 and a mixture of such probes.

10 25. Use of a peptide nucleic acid probe according to claim 1 or a mixture thereof for detecting a target sequence of one or more mycobacteria optionally present in a sample.

26. Use of a peptide nucleic acid probe or a mixture thereof according to claim 25 for detecting a target sequence of one or more mycobacteria of the Mycobacterium tuberculosis Complex  
 15 (MTC), in particular a target sequence of *M. tuberculosis*.

27. Use of a peptide nucleic acid probe or a mixture thereof according to claim 25 for detecting a target sequence of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex, in particular a target sequence of one or more  
 20 mycobacteria of the Mycobacterium avium Complex.

28. Method for detecting a target sequence of one or more mycobacteria optionally present in a sample comprising

25 (1) contacting any rRNA or rDNA present in said sample with one or more peptide nucleic acid probes according to claim 1 or a mixture thereof under conditions, whereby hybridisation takes place between said probe(s) and said rRNA or rDNA, and

30 (2) observing or measuring any formed detectable hybrids, and relating said observation or measurement to the presence of a target sequence of one or more mycobacteria in said sample.

29. Method according to claim 28 for detecting a target sequence of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC), in particular a target  
 35 sequence of *M. tuberculosis*.

30. Method according to claim 28 for detecting a target sequence of one or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex.



31. Method according to claim 28, wherein the hybridisation takes place in situ.

32. Method according to claim 28, wherein the hybridisation takes place in vitro.

5 33. A method according to claim 28,  
c h a r a c t e r i s e d in that a signal amplifying system is used for measuring the resulting  
hybridisation.

34. Method according to c l a i m 28, wherein the sample is a sputum sample.  
10

35. Kit for detecting a target sequence of one or more mycobacteria, in particular a target  
sequence of one or more mycobacteria of the Mycobacterium tuberculosis Complex (MTC), in  
particular a target sequence of M. tuberculosis, and/or for detecting a target sequence of one  
or more mycobacteria other than mycobacteria of the Mycobacterium tuberculosis Complex  
15 (MOTT), in particular a target sequence of one or more mycobacteria of the Mycobacterium  
avium Complex,  
c h a r a c t e r i s e d in that said kit comprises at least one peptide nucleic acid probe  
according to claim 1, and optionally a detection system with at least one detecting reagent.

20 36. Kit according to claim 35,  
c h a r a c t e r i s e d in that it further comprises a solid phase capture system.